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Abbreviations

ABA	Absolute bioavailability
AIC	Akaike's Information Criterion
AUC	Area under curve

CDC	Centers for Disease Control and Prevention
EDTA	Ethylenediamine tetra-acetic acid
La_2O_3	Lanthanum oxide
M	Metal
Pb	Lead
$PbCO_3$	Lead carbonate
PbO	Lead oxide
PbS	Lead sulfide
$PbSO_4$	Lead sulfate
PE	Performance Evaluation
QC	Quality Control
RBA	Relative bioavailability
RLM	Relative lead mass
U.S. EPA	United States Environmental Protection Agency
v	Volume
w	Weight

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Abstract

This report summarizes the results of a series of studies that measured the relative bioavailability (RBA) of lead in a variety of soil and soil-like test materials. Reference material (lead acetate) or lead-contaminated soils were administered orally to juvenile swine twice a day for 15 days. Blood samples were collected from each animal at multiple times during the course of the study, and samples of liver, kidney, and bone were collected at sacrifice. All samples were analyzed for lead. The RBA of a test material was estimated by fitting mathematical models to the dose-response curves for each measurement endpoint and finding the ratio of doses that gave equal responses. The final RBA for a test material was the simple average of the four endpoint-specific RBA values. Results from 19 different test materials reveal that there is a wide range of RBA values across different exposure materials, ranging from 6% to 105%. This variability in RBA between different samples highlights the importance of reliable RBA data to help improve risk assessments for lead in soil. Although the RBA value for a sample depends on the relative amounts of the different chemical and physical forms of lead present, data are not yet adequate to allow reliable quantitative predictions of RBA from chemical speciation data alone.

Introduction

Reliable evaluation of the potential hazard to children from ingestion of lead in the environment depends in part on accurate information on the rate and extent of lead absorption ("bioavailability") from each exposure medium. This is especially true for soil, since lead in soil can exist in a variety of different mineral forms and particle types, some of which tend to have low absorbability. Thus, equal ingested doses of different forms of lead in soil may not be of equal health concern.

Oral bioavailability of lead in a particular medium may be expressed either in absolute terms (absolute bioavailability) or in relative terms (relative bioavailability). Absolute bioavailability (ABA) is the fraction of lead which reaches the systemic circulation following oral ingestion. Typically, ABA is measured by comparing the time course of absorption following both oral and intravenous doses and comparing the area under the blood lead concentration vs. time curves (AUC):

$$ABA = \frac{AUC_{oral} / Dose_{oral}}{AUC_{IV} / Dose_{IV}} \quad [1]$$

This ratio is also referred to as the oral absorption fraction. Relative bioavailability (RBA) is the ratio of the ABA of lead present in some test material compared to the ABA of lead in some appropriate reference material:

$$RBA = \frac{ABA_{test}}{ABA_{reference}} \quad [2]$$

Usually the form of lead used as a reference material is a soluble compound, such as lead acetate, that is expected to completely dissolve in gastrointestinal fluids when ingested.

We have been engaged in a multi-year investigation of lead absorption in juvenile swine following oral exposure to a variety of different environmental media, especially soils and solid wastes associated with mining, milling, and smelting sites. Initial studies in the program (referred to as "Phase 0" and "Phase I") were performed by Dr. Robert Poppenga and Dr. Brad Thacker at Michigan State University (Weis et al. 1995). The study designs and protocols developed during the early studies were refined and standardized by Dr. Stan Casteel at the University of Missouri, Columbia, and applied to a number of different test materials collected from various Superfund sites. This series of measurements is collectively referred to as "Phase II," and the results are presented in this report. A more detailed presentation of the Phase II work, including raw data from all studies, is available from the United States Environmental Protection Agency (U.S. EPA) (U.S. EPA 2006). Drexler and Brattin (2006) compare the results of the Phase II *in vivo* studies with the results of an *in vitro* technique for estimating lead RBA in soil samples.

Materials and Methods

Animals. Juvenile swine were selected for use in this program because the juvenile swine is believed to be a good model for the gastrointestinal system of a human child (Weis et al. 1995, USEPA 2006). All animals were intact males of the Pig Improvement Corporation genetically defined Line 26, purchased from Chinn Farms, Clarence, Missouri. Animals were usually purchased at age 4 to 5 weeks (weaning occurs at age 3 weeks). In general, about 10% more pigs were purchased than that required for the experimental design. All animals were held under quarantine for one week to observe their health in order to allow for culling of any sick animals. In addition, to minimize weight variations between animals and groups, extra animals that were

most different in body weight (either heavier or lighter than average) four days before exposure began were also excluded from the study. The remaining animals were assigned to dose groups at random (typically 5 animals per group). When exposure began (day 0), the animals were about 5 to 6 weeks old and weighed an average of about 8 to 11 kg.

All animals were housed in individual stainless steel cages. Each animal was examined by a certified veterinary clinician (swine specialist) prior to being placed on study, and was examined daily by an attending veterinarian while on study. Blood samples were collected by venipuncture for clinical chemistry and hematological analysis on days 4, 7, and 15 to assist in clinical health assessments. Any animal that became ill and could not be promptly restored to good health by appropriate treatment was removed from the study. All animals were treated humanely and with regard for alleviation of suffering.

Diet. Animals provided by the supplier were weaned onto standard pig chow purchased from MFA Inc., Columbia, Missouri. In order to minimize lead exposure from the diet, the animals were gradually transitioned from the MFA feed to a special low-lead feed (guaranteed less than 0.2 mg/kg lead, purchased from Zeigler Brothers, Inc., Gardners, Pennsylvania) over the time interval from day -7 to -3; this low-lead feed was then provided for the duration of the study. The feed was nutritionally complete and met all requirements of the National Institutes of Health–National Research Council for swine rations. Periodic analysis of feed samples during this program indicated the mean lead level was less than the detection limit (0.05 mg/kg), corresponding to a daily intake of less than 2.5 μg/kg-day.

Each day every animal was given an amount of feed equal to 5% of the mean body weight of all animals on study. Feed was administered in two equal portions of 2.5% of the mean body weight at each feeding. Feed was provided at 11:00 AM and 5:00 PM daily.

Drinking water was provided *ad libitum* via self-activated watering nozzles within each cage. Periodic analysis of samples from randomly selected drinking water nozzles indicated the mean lead concentration in water was less than 2 μg/L, corresponding to a daily intake of less than 0.2 μg/kg-day.

Test Materials. Table 1 describes the Phase II test materials for which RBA was measured in this program and provides the analytical results for lead. As seen, 17 different samples from eight different sites were investigated, along with one sample of paint flakes mixed with clean soil and one sample of finely-ground native galena mixed with clean soil. Prior to analysis and dosing, all samples were dried (<40°C) and sieved; only materials that passed through a 60-mesh screen (corresponding to particles smaller than about 250 μm) were used, with the exception of the two samples from Study 5 (Test Materials 7 and 8), which were sieved to 150 μm. This is because it is believed that soil particles less than about 250 μm are most likely to adhere to the hands and be ingested by hand-to-mouth contact, especially in young children, and small particles may tend to have a higher absorption rate than large particles.

Each sample of test material that was evaluated in the swine bioassay program was thoroughly characterized with regard to mineral phase, particle size distribution, and matrix association using electron microprobe analysis. The relative lead mass (RLM) in each phase is the length-weighted fraction of the total lead in a sample that is present in a particular phase *i,* calculated by summing across all particles in phase *i* as follows:

$$RLM_i = \frac{\sum (L \cdot \delta \cdot F)_{phase\ i}}{\sum (L \cdot \delta \cdot F)_{all\ phases}} \quad [3]$$

where:

RLM_i = relative lead mass in phase *i*

L = longest dimension of the particle

δ = density of the particle

F = fraction (by mass) of lead in the particle

Dosing. A typical study consisted of 10 dose groups. Dose Group 1 usually consisted of 3 or 5 animals which were not exposed to any exogenous lead (control group); all other dose groups consisted of 5 animals per group. Dose Groups 2, 3, and 4 were exposed to lead acetate, usually at doses of 25, 75, or 225 μg/kg-day. These dose levels were based on experience from Phase 0 and Phase I investigations, which indicated that doses of lead acetate in the range of 25 to 675 μg Pb/kg-day gave clear and measurable increases in lead levels in all endpoints measured (blood, liver, kidney, bone). Animals in Dose Groups 5, 6, and 7 were exposed to Test Material 1, and animals in Dose groups 8, 9, and 10 were exposed to Test Material 2. The doses of test materials were usually set somewhat higher than for lead acetate (e.g., 75, 225, and 675 μg Pb/kg-day) so that measurable responses would still be likely even if the test material had a relatively low RBA. Depending on the concentration of lead in the test material and the target dose level for lead, soil intake rates needed to achieve target lead doses were usually in the range of 0.5 to 2.5 g/day.

Animals were exposed to lead acetate or test material for 15 days, with the dose for each day being administered in two equal portions given at 9:00 AM and 3:00 PM (two hours before feeding). These exposure times were selected so that lead ingestion would occur at a time when the stomach was largely or entirely empty of food. This is because the presence of food in the stomach is known to reduce lead absorption (e.g., Blake et al. 1983; Chamberlain et al. 1978; Heard and Chamberlain 1982; James et al. 1985; Rabinowitz et al. 1980).

Dose material (lead acetate or test material) was placed in the center of a small portion (about 5 grams) of moistened feed. This "doughball" was administered to the animals by hand. Dose calculations were based on measured group mean body weights and were adjusted every three days to account for animal growth. In most cases, the animals readily ingested the doughball, but occasionally an animal refused or dropped the dose. In this event, the date and amount of the missed dose were recorded and the time-weighted average dose calculation for each animal was adjusted downward accordingly.

Sample Collection and Analysis. Samples of blood were collected from each animal three or four days before exposure began, on the first day of exposure (day 0), and on multiple days thereafter (usually days 1, 2, 3, 5, 7, 9, 12, and 15). All blood samples were collected by venipuncture of the anterior vena cava and were placed immediately in purple-top Vacutainer® tubes containing calcium-EDTA (ethylenediamine tetra-acetic acid) as anticoagulant, and stored under refrigeration until analysis. Blood samples were collected each sampling day beginning at 8:00 AM, approximately one hour before the first of the two daily exposures to lead on the sampling day and 17 hours after the last lead exposure the previous day. This blood collection time was selected because the rate of change in blood lead resulting from the preceding exposures is expected to be relatively small after this interval (LaVelle et al. 1991; Weis et al. 1993).

One mL of whole blood was removed from the purple-top Vacutainer and added to 9.0 mL of "matrix modifier," a solution recommended by the Centers for Disease Control and Prevention (CDC) for analysis of blood samples for lead (CDC 2001). The composition of the matrix modifier is 0.2% (v/v) ultrapure nitric acid, 0.5% (v/v) Triton X-100, and 0.2% (w/v)

(0.015 molar) dibasic ammonium phosphate in deionized and double-distilled water. Samples of the matrix modifier were routinely analyzed for lead to ensure the absence of lead contamination.

Following collection of the final blood sample at 8:00 AM on day 15, all animals were humanely euthanized and samples of liver (posterior lobe), kidney (both sides), and bone (the right femur) were removed and stored frozen in plastic bags for lead analysis.

One gram of soft tissue (liver or kidney) was placed in a screw-cap Teflon container with 2 mL of Optima grade concentrated (70%) nitric acid and heated in an oven to 90°C overnight. After cooling, the digestate was transferred to a clean 10 mL volumetric flask and diluted to volume with deionized and double-distilled water.

The right femur of each animal was defleshed and dried at 100°C overnight. The dried bones were then broken in half, placed in a muffle furnace and dry-ashed at 450°C for 48 hours. Following dry ashing, the bone was ground to a fine powder using a mortar and pestle and 200 mg were removed and dissolved in 10.0 mL of 1:1 (v:v) Optima grade concentrated nitric acid/water. After the powdered bone was dissolved and mixed, 1.0 mL of the acid solution was removed and diluted to 10.0 mL by addition of 0.1% (w/v) lanthanum oxide (La_2O_3) in deionized and double-distilled water.

Samples of biological tissue (blood, liver, kidney, bone) and other materials (e.g., food, water, reagents, solutions) were arranged in a random sequence and provided to U.S. EPA's analytical laboratory in a blind fashion (identified to the laboratory only by a chain of custody tag number). Each sample was analyzed for lead using a PerkinElmer (Wellesley, Massachusetts) Model 5100 graphite furnace atomic absorption spectrophotometer. Internal quality control (QC) samples were run every tenth sample and the instrument was recalibrated every 15^{th} sample. A blank, duplicate, and spiked sample were run every 20^{th} sample.

All results from the analytical laboratory were reported in units of μg Pb/L of prepared sample. The detection limit was defined as three-times the standard deviation of a set of seven replicates of a low-lead sample (typically about 2 to 5 μg/L). The standard deviation was usually about 0.3 μg/L, so the detection limit was usually about 0.9 to 1.0 μg/L. However, because different dilution factors were used for different sample types, the detection limit varies from sample type to sample type. For prepared blood samples (diluted 1/10), this corresponds to a detection limit of 10 μg/L (1 μg/dL). For soft tissues (liver and kidney, also diluted 1/10), this corresponds to a detection limit of 10 μg/kg wet weight. For bone (final dilution of 1/500), the corresponding detection limit is 0.5 μg/g ashed weight.

Quality Assurance. A number of steps were taken throughout each of the studies in this program to assess and document the quality of the data that were collected. These steps are summarized below.

Duplicates: A randomly selected set of about 5% of all blood and tissue samples generated during each study were submitted to the laboratory in a blind fashion for duplicate analysis. There was good reproducibility between duplicate samples for both blood and tissues, with both linear regression lines having a slope near 1.0, an intercept near zero, and an R^2 value near 1.00.

Performance Standards for Blood: Three sets of performance evaluation (PE) blood samples were obtained from CDC, with nominal concentrations of 1.7 μg/dL, 4.8 μg/dL, and 14.9 μg/dL. Each day that blood samples were collected from experimental animals, several PE samples of different concentrations were also prepared and submitted for analysis in random order and in a blind fashion. Analytical results obtained for the PE samples were generally in good agreement with the expected value at all three concentrations, with an overall mean of 1.4

μg/L for the low standards (nominal concentration of 1.7 μg/L), 4.3 μg/L for the middle standard (nominal concentration of 4.8 μg/L), and 14.5 μg/L for the high standards (nominal concentration of 14.9 μg/L).

Interlaboratory Comparison: In each study, an interlaboratory comparison of blood lead analytical results was performed by sending a set of about 15 to 20 randomly selected whole blood samples to CDC for blind independent preparation and analysis. The results from U.S. EPA's laboratory were generally similar to those of CDC, with a mean inter-sample difference (U.S. EPA minus CDC) of 0.07 μg/dL. The slope of the best-fit straight line through the paired data was 0.84, indicating that the concentration values estimated by the U.S. EPA laboratory tended to be about 15% lower than those estimated by CDC. The reason for this apparent discrepancy between the U.S. EPA laboratory and the CDC laboratory is not clear, but might be related to differences in sample preparation techniques. Regardless of the reason, the differences are sufficiently small that they are likely to have no significant effect on calculated RBA values. In particular, it is important to realize that if both the lead acetate and test material dose-response curves are biased by the same factor, then the biases cancel in the calculation of the ratio.

Approach for Estimating RBA. The method used to estimate the RBA of lead in a particular test material compared to the reference material (lead acetate) is based on the principal that equal absorbed doses of lead will produce equal biological responses. By definition:

$$\textit{Absorbed dose (reference)} = \textit{Administered dose (reference)} \cdot \textit{ABA (reference)} \quad [4]$$

$$\textit{Absorbed dose (test)} = \textit{Administered dose (test)} \cdot \textit{ABA (test)} \quad [5]$$

When responses are equal, then absorbed doses are equal, and:

$$\textit{Administered dose (reference)} \cdot \textit{ABA (reference)} = \textit{Administered dose (test)} \cdot \textit{ABA (test)}$$

Thus:

$$RBA = \frac{ABA\,(test)}{ABA\,(reference)} = \frac{Administered\ dose\,(reference)}{Administered\ dose\,(test)} \quad [6]$$

That is, given the dose-response curve for some particular endpoint (e.g., blood lead AUC or the concentration of lead in liver, kidney or bone) for both the reference material and the test material, RBA may be calculated as the ratio of administered doses that produce equal biological responses (and not as the ratio of responses at equal doses). Note that, in this approach, the mathematical form of the dose-response model must be the same for both reference material and test material. This is because the shape of the dose-response curve is a function only of the pharmacokinetic response of the biological organism to an absorbed dose of lead, and the response per unit absorbed dose does not depend on whether the absorbed lead was derived from reference material or test material.

Statistical Methods for Fitting Dose-Response Models. The techniques used to derive statistical models of the dose-response data and to estimate RBA are based on the methods recommended by Finney (1978). All model fitting was performed using JMP® version 3.2.2, a commercial software package developed by SAS®.

As noted by Finney (1978), when the data to be analyzed consist of two or more dose-response curves from the same study (e.g., lead acetate, test material 1, test material 2), it is apparent that all curves must have the same intercept, since there is no difference between the curves when the dose is zero. This requirement is achieved by fitting all of the data from a study simultaneously, and requiring the intercept to be identical for each curve.

Regression analysis based on ordinary least squares minimization assumes that the variance of the responses is independent of the dose and/or the response (Draper and Smith 1998). In these studies, this assumption is generally not satisfied, as variability in response tends to increase as a function of increasing dose. This is referred to as heteroscedasticity. One

method for dealing with heteroscedasticity is through the use of weighted least squares regression (Draper and Smith 1998). In this approach, each observation in a group of animals is assigned a weight that is inversely proportional to the variance of the response in that group:

$$w_i = \frac{1}{\sigma_i^2} \qquad [7]$$

where:

w_i = weight assigned to all data points in dose group i

σ_i^2 = variance of responses in animals in dose group i

We considered several options for estimating the value of σ_i^2 , including:

Option 1: Utilize the observed variance (s_i^2) in the responses of animals in dose group i.

Option 2: Establish a variance model of the form $\sigma_i^2 = \alpha\mu_i^{\rho}$, where μ_i is the predicted mean response for dose group i. Simultaneously fit the data to derive values of α and ρ along with the other coefficients of the dose-response model using the data from a particular study. This approach is identical to the non-constant variance approach used by U.S. EPA's Benchmark Dose Software (U.S. EPA 1995, 2001).

Option 3a: Establish an "external" variance model based on an analysis of the relationship between variance and mean response using observations combined from all studies and dose groups. Use that model to predict the expected variance in dose group i as a function of the predicted mean response (i.e., the mean response predicted from the best-fit equation through the dose-response data) for that dose group.

Option 3b: Establish an "external" variance model based on an analysis of the relationship between variance and mean response using observations combined from all studies and dose groups. Use that model to predict the expected variance in dose group i as a function of

the observed mean response level (i.e., the mean response measured in the exposed animals) for that dose group.

Based on a consideration of the advantages and disadvantages of each approach, Option 3b was selected for use in this project. This is mainly because it has relatively less vulnerability than other options to random variations in observed variances in a dose group (which results in assignment of weights that are either too high or too low). Option 3b was preferred over Option 3a because Option 3a is based on predicted mean response while Option 3b is based on observed mean response. It should be noted, however, that Option 3b is somewhat vulnerable to poor fits when one particular dose group in a data set lies well below the expected smooth fit through the other dose groups. In this case, the variance assigned to the group (based on the observed mean response) is lower than typical for that dose level (and hence the weights assigned to the data are higher than usual), tending to force the line through that data set at the expense of the other data sets.

The external variance model for Option 3b was based on the consolidated data from all studies. In this analysis, some dose groups were excluded if the estimate of variance and/or mean response was judged to be unreliable, based on the following two criteria: a) the number of animals in the dose group was less than 3, or b) the fraction of responses below the detection limit was more than 20%. Figure 1 shows the log-variance in response plotted as a function of the log-mean response in the group. One panel is presented for each of the four different endpoints. As seen, log-variance increases as an approximately linear function of log-mean response for all four endpoints:

$$\ln(s_i^{\,2}) = k1 + k2 \cdot \ln(\bar{y}_i) \qquad [8]$$

where:

$\bar{y}_i$ = mean observed response of animals in dose group *i*

Values of *k1* and *k2* were derived from the data for each endpoint using ordinary least squares minimization. The resulting values are shown in Table 2. Based on these variance models, the weights for each response in a dose group were assigned based on the observed mean response for that dose group:

$$\sigma_i^2 = \exp[k1 + k2 \cdot \ln(\bar{y}_i)] \quad [9]$$

Choice of Model Forms. As noted above, the main objective of the curve-fitting effort is to find a mathematical model that fits both the reference and test group dose-response data sets smoothly. Note that there is no requirement that the model have a mechanistic basis or that the coefficients have a biological meaning. As discussed by Finney (1978), it is generally not appropriate to choose the form of the dose-response model based on only one experiment, but to make the choice based on the weight of observations across many different studies. Four different models were evaluated, including:

1. Linear: $y = a + bx$ [10]

   $RBA = b(test) / b(reference)$ [11]

2. Exponential: $y = a + b \cdot (1 - \exp(-cx))$ [12]

   $RBA = c(test) / c(reference)$ [13]

3. Michaelis-Menton: $y = a + bx / (c + x)$ [14]

   $RBA = c(reference) / c(test)$ [15]

4. Power: $y = a + bx^c$ [16]

$$RBA = [b(test) / b(reference)]^{1/c} \quad [17]$$

For each data set, the preferred model was identified based on Akaike's Information Criterion (AIC) (U.S. EPA 2000, 2001). Based on fitting each dose-response data set to each of the four models above, it was found that the linear model most frequently gave the best fit for liver, kidney, and bone. In the few cases where the linear model was not the best fit, the RBA value given by the linear model was usually close to that given by whatever other model did provide the best fit. On this basis, the linear model was selected for application to all dose-response data sets for liver, kidney, and bone.

For the blood lead AUC endpoint, the linear model usually gave the worst fit, and on this basis it was rejected as a candidate for the AUC endpoint. In general, each of the three nonlinear models (exponential, Michaelis-Menton, and power) all tended to give similar results in terms of RBA value (the standard deviation in RBA for a particular test material averaged across the three models was usually less than 3%) and differences in the AIC were usually small. On this basis, it was concluded that any of these three models would be acceptable. The power model was not selected because it does not tend toward a plateau, while data from early blood lead pilot studies (using higher doses than commonly used in the Phase II studies) suggest that the blood lead endpoint does tend to do so. Of the remaining two models (exponential and Michaelis-Menton), the exponential model was selected mainly because it yielded the best fit more often than the Michaelis-Menton model and because the exponential model had been used in previous analyses

of the data. Thus, the exponential model was selected for application to all dose-response data sets for the blood AUC endpoint, except in one special case, noted below.

In Study 7 (Test Materials 11 and 12), the blood lead AUC data set did not yield a solution in JMP for the exponential model, probably because the data have relatively less curvature than most blood lead AUC data sets. Because of this lack of curvature, it was not possible to estimate the exponential plateau value (b) with confidence, which in turn made it difficult to estimate the other parameters of the exponential model. Several alternative approaches for data reduction were evaluated, including a) using the model fits from one of the other nonlinear models, b) using the fit for the linear model, and c) fitting the data to the exponential model using a defined value for the plateau based on results from other data sets. The results (i.e., the RBA values based on the blood lead AUC endpoint) were generally similar for all three of these approaches, so the results from the linear fit were used.

Assessment of Outliers: For the purposes of this program, endpoint responses that yielded standardized weighted residuals greater than 3.5 or less than -3.5 were considered to be potential outliers (Canavos 1984). A total of 13 such cases occurred out of a total of 1,895 endpoint responses (0.7%). In these cases, RBA values were calculated both with and without the outliers. In most cases there was very little difference (the average ratio of RBA with outlier excluded to RBA with outlier included was 1.09). All results presented here are based on the analysis with outliers excluded.

Uncertainty Bounds in Endpoint-Specific RBA Values. The uncertainty bounds around each endpoint-specific RBA value were estimated based on Fieller's Theorem, as described by Finney (1978).

Combination of RBA Estimates across Endpoints. As discussed above, each study of RBA utilized four different endpoints to estimate absorption of lead, including blood AUC, liver, kidney, and bone. Consequently, each study yielded four independent endpoint-specific estimates of RBA for each test material. Thus, the final RBA estimate for a test material involves combining the four endpoint-specific RBA values into a single value (point estimate) and estimating the uncertainty around that point estimate. The basic strategy selected for deriving a point estimate of RBA for a test material was to calculate a confidence-weighted average of the four endpoint-specific RBA values. Because each endpoint-specific RBA value is calculated as the ratio of the parameters of the dose-response curves fitted to the experimental data for reference material and test material, the relative confidence in an endpoint-specific RBA is inherently related to the quality of the data that define the dose-response curve for that endpoint. Thus, the indicator we selected to quantify the relative reliability of the four different endpoints is the magnitude of the uncertainty (standard error) around RBA estimates based on each endpoint.

Figure 2 plots the standard error in each RBA estimate as a function of the RBA value for each of the four different endpoints. As seen, uncertainty in RBA (as reflected in the magnitude of the standard error) increases as a function of the estimated value of RBA for all four endpoints. This is expected because of the heteroscedasticity in the underlying dose-response data. Although RBA values based on blood AUC or femur tend to yield estimates with slightly lower standard errors than RBA values based on liver or kidney, the magnitude of the standard errors tends to be generally similar for all four endpoints and the difference between the four regression lines is not statistically significant ($p = 0.699$). Based on this, each endpoint-specific

RBA value was judged to have approximately equal validity, so the point estimate was calculated as the simple average across all four endpoint-specific RBA values.

The uncertainty bounds around each point estimate were estimated using Monte Carlo simulation. Each endpoint-specific RBA uncertainty distribution was assumed to be normal, with the mean equal to the best estimate of RBA and the standard error estimated from Fieller's Theorem. In the Monte Carlo simulation, a value was drawn from one of the four uncertainty distributions, with an equal probability of choosing each of the distributions. The uncertainty in the point estimate was characterized as the range from the 5^{th} to the 95^{th} percentile of these random values.

Results

Dosing Effects on Animal Health and Weight. Lead dose levels employed in this program were substantially below levels that cause clinical symptoms in swine and no evidence of treatment-related toxicity was observed in any dose group. All animals exposed to lead by the oral route remained in good health throughout each study; the only clinical signs observed were characteristic of normal swine. Animals typically gained about 0.3 to 0.5 kg/day, and the rate of weight gain was normally comparable in all exposure groups.

Time Course of Blood Lead Response. Figure 3 presents an example graph of the time course of pseudo-steady-state blood lead levels following repeated oral exposure to lead acetate. As seen, blood lead levels begin below the quantitation limit (usually about 1 μg/dL) and stay very low in control animals throughout the course of the study. In animals exposed to lead acetate, blood lead values begin to rise within 1 to 2 days and tend to flatten out to a near steady

state within about 7 to 10 days. The temporal pattern is similar for test materials that are absorbed well enough to provide a clear response.

Dose-Response Patterns. Figures 4 to 7 present the dose-response patterns observed for blood, liver, kidney, and bone (femur) following repeated oral exposure to lead acetate. For blood, the endpoint is the area under the blood lead *vs.* time curve (AUC). For femur, kidney, and liver, the endpoint is the concentration in the tissue at the time of sacrifice. The data are based on the combined results across all studies performed during Phase II.

As seen, there is substantial variability in response between individuals (both within and between studies), and this variability tends to increase as dose (and response) increases. As noted above, this pattern of increasing variance in response (heteroscedasticity) is accounted for in the model-fitting procedure through the use of weighted least squares regression. Despite the variability in response, it is apparent that the dose-response pattern is typically non-linear for blood lead AUC, but is approximately linear for liver, kidney, and bone lead. This pattern of dose-response relationships suggests that, at least over the dose range tested in this program, absorption of lead from the gastrointestinal tract of swine is linear and that the non-linearity observed in blood lead AUC response is due to saturable binding in the blood compartment. This conclusion is based on the logic that, if the non-linear behavior observed for blood were due to non-linear absorption from the gastrointestinal tract, it would be extremely unlikely that all three of the other endpoints observed (liver, kidney, bone) would respond linearly.

Characterization of Test Materials. Table 3 lists the different lead phases observed in the test materials. Note that only a few of the phases are stoichiometric minerals (anglesite = $PbSO_4$, cerussite = $PbCO_3$, galena = PbS, native lead = Pb), while the others are non-stoichiometric associations of various metals (M) and other elements. As shown in Table 3, of the 22 different

phases observed in one or more samples, nine are very minor, with RLM values no higher than 2% in any sample. However, 13 of the phases occur at concentrations that could contribute significantly to the overall bioavailability of the sample (RLM > 10%). It should be noted that a lead-bearing particle that is present in a bulk sample from a slag pile is classified as slag only if the particle is glassy or vitreous in nature. Inclusions or other non-vitreous grains of lead-bearing material that may be present are classified according to their mineral content (e.g., lead oxide, galena).

Table 4 summarizes information on the degree to which lead-bearing particles in each sample are partially or entirely liberated (i.e., exposed to gastric fluids when ingested) or included (i.e., fully enclosed or encased in mineral or vitreous matrices). Data are presented both on a particle frequency basis and on the basis of relative lead mass. As seen, the majority of lead-bearing particles in most samples are partially or entirely liberated, although Test Material 19 (Oregon Gulch tailings) is a clear exception. Table 5 summarizes data on the frequency distribution of particle sizes (measured as the longest dimension) in each sieved sample. For convenience, the data presented are for liberated particles only. As seen, most samples contain a range of particle sizes, often with the majority of the particles being less than 50 μm long.

RBA Results for Test Materials. Endpoint-specific RBA estimates for each test material are summarized in Table 6. The final point estimate for each test material is shown in the right-hand column. Inspection of these point estimates for the different test materials reveals that there is a wide range of values across different samples, both within and across sites. For example, at the California Gulch site in Colorado, RBA estimates for different types of material range from about 6% (Test Material 19, Oregon Gulch tailings) to about 105% (Test Material 12, Fe/Mn lead oxide sample). This wide variability highlights the importance of obtaining and applying

reliable RBA data to site-specific samples in order help to improve risk assessments and more efficiently focus risk management of childhood lead exposure.

Reproducibility. Only one sample (Test Material 14, Palmerton Location 2) was analyzed in duplicate during the Phase II study. As seen, agreement is moderately good between the two studies for the blood AUC and kidney endpoints and for the point estimate, although there is relatively low agreement for the liver and bone endpoints.

Correlation of RBA with Mineral Phase. In principle, each unique combination of phase, size, and matrix association constitutes a unique mineralogical form of lead, and each unique form could be associated with a unique RBA that is the inherent value for that "type" of lead. If so, then the expected RBA value observed for a sample containing a mixture of different "types" of lead is the concentration-weighted average across all of the unique forms present in the sample. If the number of different lead phases which may exist in the environment is on the order of 20 or more, the number of size categories is on the order of five, and the number of matrix association categories is two (included, liberated), then the total number of different "types" of lead is on the order of 200 or more. Because measured RBA data are available from this study for only 19 different samples, it is clearly impossible with the present data set to estimate "type-specific" RBA values for each combination of phase, size, and matrix association. Therefore, in order to simplify the analysis process, it was assumed that the measured RBA value for a sample was dominated by the liberated mineral phases present and the effect of included materials and particle size were not considered. That is, the data were analyzed according to the following model:

$$RBA_{sample} = \sum_{i=1}^{p} (C_{i,liberated} \cdot RBA_{i,liberated}) \qquad [18]$$

where:

RBA_{sample} = observed RBA of lead in a sample

$C_{i,liberated}$ = fraction of total lead in liberated particles of phase *i*

$RBA_{i,liberated}$ = RBA of lead in liberated particles of phase *i*

p = number of different lead phase categories

Because 22 different phases were identified and only 19 different samples were analyzed, it was necessary to reduce the number of phases to a smaller number so that regression analysis could be performed. Therefore, the different phases were grouped into ten categories as shown in Table 7. These groups were based on professional judgment regarding the expected degree of similarity among the different phases, along with information on the relative abundance of each phase (see Table 3). The total lead mass in each phase grouping was calculated by summing the relative lead mass for each individual component in the group. As noted above, only the lead mass in partially or entirely liberated particles was included in the sum. Group-specific RBA values were estimated by fitting the grouped data to the model using minimization of squared errors. Each parameter was constrained to be greater than or equal to zero. Because Group 10 contains only phases that are present in relatively low levels, an arbitrary coefficient of 0.5 was assumed for this group and the coefficient was not treated as a fitting parameter.

The resulting estimates of the group-specific RBA values are shown in Figure 8. As seen, there is a wide range of group-specific RBA values. It is important to stress that these group-specific RBA estimates are derived from a very limited data set (nine independent parameter estimates based on only 19 different measurements), so the group-specific RBA estimates are inherently uncertain. In addition, both the measured sample RBA values and the relative lead mass in each phase are subject to additional uncertainty. Therefore, the group-

specific RBA estimates should not be considered highly precise and calculation of a quantitative sample-specific RBA value from these estimates is not appropriate. Rather, it is more appropriate to consider the results of this analysis as sufficient to support only semi-quantitative (low, medium, high) classification of phase-specific RBA values. As noted above, the estimates apply only to particles that are liberated, not those that are included.

Conclusions

Juvenile swine are believed to be a useful model of gastrointestinal absorption in children. The results from the studies conducted during this program indicate that juvenile swine can be used to measure lead RBA in a variety of soil-like test materials. Each RBA estimate is uncertain due to the variability in response between different animals, but the magnitude of this uncertainty can be quantified to allow risk managers flexibility in choosing a value for use in risk assessment and risk management decision-making. If necessary, the magnitude of the uncertainty can be reduced by using either more animals per dose group and/or more dose groups to help define the dose-response curves with greater certainty.

Each of the four different endpoints employed in these studies (blood AUC, liver, kidney, bone) to estimate RBA appear to yield reasonable values, with no one endpoint being clearly superior to the others. Thus, the best estimate of the RBA value for any particular sample is the average across all four endpoint-specific RBA values, and combining results from the independent endpoints helps increase confidence in the point estimate.

There are clear differences in the RBA of lead between different types of test material, ranging from near zero to close to 100%. Thus, reliable data on the RBA value for different types of test materials at a site can be very important in improving lead risk assessments at a site.

The U.S. EPA default value for the RBA of lead in soil is 60% (U.S. EPA 1994). Of the 17 authentic site soil samples tested in this program, eight had point estimate values within 20% of the default (i.e., from 40% to 80%), six had point estimate RBA values less than 40% and three had point estimate values greater than 80%. Thus, based on this set of samples, the EPA default value of 60% appears to be a reasonable central tendency value.

Presumably, the RBA value for any one sample is a weighted function of the "phase-specific" RBA values for each lead phase present in the sample. Available data support the view that certain types of lead minerals are well absorbed (e.g., cerussite, manganese lead oxide), while other forms are poorly absorbed (e.g., galena, anglesite). However, the data are not yet sufficient to allow reliable quantitative calculation or prediction of the RBA for a test material based on knowledge of the lead mineral content alone.

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Table 1. Description of Phase II test materials.

Test Material Number	Study	Sample Designation	Site	Sample Description	Lead Concentration (ppm) [a]
1	2	Bingham Creek Residential	Kennecott NPL Site, Salt Lake City, Utah	Soil composite of samples containing less than 2500 ppm lead; collected from a residential area (Jordan View Estates) located along Bingham Creek in the community of West Jordan, Utah.	1,590
2	2	Bingham Creek Channel Soil	Kennecott NPL Site, Salt Lake City, Utah	Soil composite of samples containing 3000 ppm or greater of lead; collected from a residential area (Jordan View Estates) located along Bingham Creek in the community of West Jordan, Utah.	6,330
3	3	Jasper County High Lead Smelter	Jasper County, Missouri Superfund Site	Soil composite collected from an on-site location.	10,800
4	3	Jasper County Low Lead Yard	Jasper County, Missouri Superfund Site	Soil composite collected from an on-site location.	4,050
5	4	Murray Smelter Slag	Murray Smelter Superfund Site, Murray City, Utah	Composite of samples collected from areas where exposed slag existed on site.	11,700
6	4	Jasper County High Lead Mill	Jasper County, Missouri Superfund Site	Soil composite collected from an on-site location.	6,940
7	5	Aspen Berm	Smuggler Mountain NPL Site, Aspen, Colorado	Composite of samples collected from the Racquet Club property (including a parking lot and a vacant lot).	14,200
8	5	Aspen Residential	Smuggler Mountain NPL Site, Aspen, Colorado	Composite of samples collected from residential properties within the study area.	3,870
9	6	Midvale Slag	Midvale Slag NPL Site, Midvale, Utah	Composite of samples collected from a water-quenched slag pile in Midvale Slag Operable Unit 2.	8,170
10	6	Butte Soil	Silver Bow Creek/Butte Area NPL Site, Butte, Montana	Soil composite collected from waste rock dumps in Butte Priority Soils Operable Unit (BPSOU).	8,530
11	7	California Gulch Phase I Residential Soil	California Gulch NPL Site, Leadville, Colorado	Soil composite collected from residential properties within Leadville.	7,510

12	7	California Gulch Fe/Mn PbO	California Gulch NPL Site, Leadville, Colorado	Soil composite collected from near the Lake Fork Trailer Park located southwest of Leadville near the Arkansas River.	4,320
13	8	California Gulch AV Slag	California Gulch NPL Site, Leadville, Colorado	Sample collected from a water-quenched slag pile on the property of the former Arkansas Valley (AV) Smelter, located just west of Leadville.	10,600
14	9	Palmerton Location 2	New Jersey Zinc NPL Site, Palmerton, Pennsylvania	Soil composite collected from on-site.	3,230
15	9	Palmerton Location 4	New Jersey Zinc NPL Site, Palmerton, Pennsylvania	Soil composite collected from on-site.	2,150
16	11	Murray Smelter Soil	Murray Smelter Superfund Site, Murray City, Utah	Soil composite collected from on-site.	3,200
17	11	NIST Paint	--	A mixture of approximately 5.8% NIST Standard Reference Material (SRM) 2589 and 94.2% low lead soil (< 50 ppm) collected in Leadville, Colorado. NIST SRM 2589, composed of paint collected from the interior surfaces of houses in the US, contains a nominal lead concentration of 10% (100,000 ppm); the material is powdered with more than 99% of the material being less than 100 µm in size.	8,350
18	12	Galena-enriched Soil	--	A mixture of approximately 1.2% galena and 98.8% low lead soil (< 50 ppm) that was collected in Leadville, Colorado. The added galena consisted of a mineralogical (i.e., native) crystal of pure galena that was ground and sieved to obtain fine particles smaller than about 65 µm.	11,200
19	12	California Gulch Oregon Gulch Tailings	California Gulch NPL Site, Leadville, Colorado	A composite of tailings samples collected from the Oregon Gulch tailings impoundment.	1,270

[a] Samples were analyzed for lead by inductively coupled plasma-atomic emission spectrometry (ICP-AES) in accord with USEPA Method 200.7. All samples were dried and sieved to 250 µm before analysis, except for the two Aspen samples (Experiment 5), which were sieved to 150 µm before analysis.

Table 2. Values for the variance model parameters, *k1* and *k2*.

Endpoint	k1	k2
Blood AUC	-1.3226	1.5516
Liver	-2.6015	2.0999
Kidney	-1.8499	1.9557
Femur	-1.9713	1.656

Table 3. Relative lead mass of mineral phases observed in test materials.

Phase	Test Material																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Anglesite	--	28%	1%	0.5%	1.0%	2%	7%	1%	--	36%	10%	--	2%	6%	4%	--	1%	--	--
As(M)O	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.003%	--	--	--
Calcite	--	--	0.2%	--	--	0.1%	--	--	--	--	--	--	--	--	--	--	--	--	--
Cerussite	2%	0.3%	32%	81%	1.1%	57%	62%	64%	4%	0.3%	20%	--	1%	--	--	14%	55%	--	--
Clay	--	--	0.018%	0.003%	--	0.017%	0.1%	--	--	0.1%	--	0.01%	--	0.03%	0.13%	--	--	--	--
Fe-Pb--Oxide	6%	3%	14%	2%	2%	10%	9%	7%	0.3%	7%	6%	8%	51%	2%	2%	0.13%	--	--	--
Fe-Pb--Sulfate	22%	30%	3%	1%	0.3%	1%	5%	5%	0.1%	20%	6%	3%	0.3%	1%	--	0.6%	--	--	--
Galena	--	9%	--	8%	9%	3%	12%	17%	6%	12%	2%	--	3%	--	--	20%	--	100%	100%
Lead--Barite	--	0.04%	--	--	--	0.01%	0.06%	--	--	0.007%	0.15%	0.14%	--	1%	0.1%	--	--	--	--
Lead--Organic	--	0.3%	--	--	--	--	0.03%	0.03%	--	--	0.11%	0.11%	1%	--	--	--	--	--	--
Lead--Oxide	--	--	0.09%	--	69%	7%	--	--	--	--	--	--	--	--	--	27%	44%	--	--
Lead--Phosphate	50%	26%	21%	6%	--	7%	1%	1%	--	3.6%	30%	15%	--	24%	1%	--	--	--	--
Lead--Silicate	--	--	--	0.04%	--	0.5%	--	--	--	--	1.9%	0.8%	--	--	1.4%	--	--	--	--
Lead--Vanidate	--	--	--	--	--	--	--	--	--	--	0.1%	0.4%	--	--	18%	--	--	--	--
Mn-Pb--Oxide	18%	2%	2%	2%	0.8%	9%	4%	5%	--	20.2%	22%	72%	--	66%	66%	--	--	--	--
Native--Lead	--	--	22%	--	0.7%	2%	--	--	15%	--	--	--	--	--	--	--	--	--	--
Pb(M)O	--	--	--	--	4%	--	--	--	26%	--	--	--	--	--	7%	3%	--	--	--
Pb-As--Oxide	2%	1%	--	0.15%	6%	--	--	--	33%	--	0.1%	--	31%	--	--	29%	--	--	--
PbO-Cerussite	--	--	--	--	--	--	--	--	--	--	1%	--	--	--	--	--	--	--	--
Slag	--	--	4%	--	7%	1%	--	--	16%	--	1%	--	10%	--	--	6%	--	--	--
Sulfosalts	--	--	--	--	--	--	--	--	0.4%	--	--	--	--	--	--	--	--	--	--
Zn-Pb--Silicate	--	--	--	--	0.03%	--	--	--	--	--	--	--	--	--	2%	--	--	--	--

M = metal

Table 4. Matrix associations of lead particles in test materials.

Test	Particle Frequency		Relative Lead Mass	
Material	Liberated	Included	Liberated	Included
1	100%	0%	100%	0%
2	100%	0%	100%	0%
3	81%	19%	76%	24%
4	100%	0%	94%	6%
5	87%	13%	77%	23%
6	96%	4%	93%	7%
7	86%	14%	93%	8%
8	98%	2%	94%	6%
9	91%	9%	77%	23%
10	91%	9%	91%	9%
11	79%	21%	65%	35%
12	98%	2%	100%	0%
13	78%	22%	80%	20%
14	100%	0%	100%	0%
15	79%	21%	89%	11%
16	80%	20%	70%	30%
17	100%	0%	100%	0%
18	100%	0%	100%	0%
19	2%	98%	5%	95%

Table 5. Length distributions for lead-bearing particles in test materials.

Test	Particle Size (μm)								
Material	<5	5-9	10-19	20-49	50-99	100-149	150-199	200-249	>250
1	38%	22%	19%	16%	4%	2%	0%	0%	0%
2	66%	13.6%	10%	6.1%	3%	1%	0%	0%	0%
3	44%	19%	8%	8%	9%	9%	2%	1%	1%
4	29%	20%	21%	20%	8%	3%	0%	0%	0%
5	14%	13%	15%	6%	20%	24%	4%	3%	0%
6	23%	21%	22%	19%	9%	6%	1%	1%	0%
7	27%	19%	22%	17%	8%	6%	1%	1%	0%
8	38%	35%	12%	8%	4%	2%	0%	0%	0%
9	6%	1%	3%	4%	20%	29%	18%	13%	5%
10	23%	15%	14%	23%	14%	9%	2%	1%	0%
11	24%	9%	18%	22%	15%	9%	1%	1%	1%
12	26%	19%	24%	17%	10%	4%	0%	0%	0%
13	19%	8%	8%	5%	9%	19%	10%	13%	9%
14	26%	23%	25%	18%	6%	1%	0%	0%	0%
15	25%	15%	21%	25%	13%	2%	0%	0%	0%
16	23%	10%	29%	17%	6%	8%	3%	3%	1%
17	76%	4%	6%	8%	6%	0%	0%	0%	0%
18	48%	2%	4%	41%	4%	0%	0%	0%	0%
19	85%	8%	6%	0%	0%	0%	0%	0%	0%

Table 6. Estimated lead RBA values for test materials.

Test	Blood AUC			Liver			Kidney			Femur			Point Estimate		
Material	RBA	LB	UB	RBA	LB	UB	RBA	LB	UB	RBA	LB	UB	RBA	LB	UB
1	0.34	0.23	0.50	0.28	0.20	0.39	0.22	0.15	0.31	0.24	0.19	0.29	0.27	0.17	0.40
2	0.30	0.20	0.45	0.24	0.17	0.34	0.27	0.19	0.37	0.26	0.21	0.31	0.27	0.19	0.36
3	0.65	0.47	0.89	0.56	0.42	0.75	0.58	0.43	0.79	0.65	0.52	0.82	0.61	0.43	0.79
4	0.94	0.66	1.30	1.00	0.75	1.34	0.91	0.68	1.24	0.75	0.60	0.95	0.90	0.63	1.20
5	0.47	0.33	0.67	0.51	0.33	0.88	0.31	0.22	0.46	0.31	0.23	0.41	0.40	0.23	0.64
6	0.84	0.58	1.21	0.86	0.54	1.47	0.70	0.50	1.02	0.89	0.69	1.18	0.82	0.51	1.14
7	0.69	0.54	0.87	0.87	0.58	1.39	0.73	0.46	1.26	0.67	0.51	0.89	0.74	0.48	1.08
8	0.72	0.56	0.91	0.77	0.50	1.21	0.78	0.49	1.33	0.73	0.56	0.97	0.75	0.50	1.04
9	0.21	0.15	0.31	0.13	0.09	0.17	0.12	0.08	0.18	0.11	0.06	0.18	0.14	0.07	0.24
10	0.19	0.14	0.29	0.13	0.09	0.19	0.15	0.09	0.22	0.10	0.04	0.19	0.14	0.06	0.23
11	0.88	0.62	1.34	0.75	0.53	1.12	0.73	0.50	1.12	0.53	0.33	0.93	0.72	0.38	1.07
12	1.16	0.83	1.76	0.99	0.69	1.46	1.25	0.88	1.91	0.80	0.51	1.40	1.05	0.57	1.56
13	0.26	0.19	0.36	0.19	0.11	0.32	0.14	0.08	0.25	0.20	0.13	0.30	0.20	0.09	0.31
14	0.82	0.61	1.05	0.60	0.41	0.91	0.51	0.30	0.91	0.47	0.37	0.60	0.60	0.34	0.93
15	0.62	0.47	0.80	0.53	0.37	0.79	0.41	0.25	0.72	0.40	0.32	0.52	0.49	0.29	0.72
16	0.70	0.54	0.89	0.58	0.42	0.80	0.36	0.25	0.52	0.39	0.31	0.49	0.51	0.29	0.79
17	0.86	0.66	1.09	0.73	0.52	1.03	0.55	0.38	0.78	0.74	0.59	0.93	0.72	0.44	0.98
18	0.01	0.00	0.02	0.02	0.00	0.04	0.01	0.00	0.02	0.01	-0.01	0.03	0.01	0.00	0.03
19	0.07	0.04	0.13	0.11	0.04	0.21	0.05	0.02	0.09	0.01	-0.04	0.06	0.06	-0.01	0.15
14R[a]	0.71	0.55	0.99	1.25	0.82	2.03	0.54	0.35	0.80	0.95	0.69	1.30	0.86	0.43	1.52

LB = 5% Lower confidence bound

UB = 95% Upper confidence bound

[a] Repeat analysis of Test Material 14

Table 7. Grouped lead phases.

Group	Group Name	Phase Constituents
1	Galena	Galena (PbS)
2	Cerussite	Cerussite
3	Mn(M) Oxide	Mn-Pb Oxide
4	Lead Oxide	Lead Oxide
5	Fe(M) Oxide	Fe-Pb Oxide (including Fe-Pb Silicate)
		Zn-Pb Silicate
6	Lead Phosphate	Lead Phosphate
7	Anglesite	Anglesite
8	Pb(M) Oxide	As(M)O
		Lead Silicate
		Lead Vanidate
		Pb(M)O
		Pb-As Oxide
9	Fe(M) Sulfate	Fe-Pb Sulfate
		Sulfosalts
10	Minor Constituents	Calcite
		Clay
		Lead Barite
		Lead Organic
		Native Lead
		PbO-Cerussite
		Slag

Figure Legends

Figure 1. External variance models.

Figure 2. Evaluation of relative precision of measurement endpoints.

Figure 3. Example time course of blood lead response.

Figure 4. Dose-response curve for blood lead AUC.

Figure 5. Dose-response curve for liver lead concentration.

Figure 6. Dose-response curve for kidney lead concentration.

Figure 7. Dose-response curve for femur lead concentration.

Figure 8. Estimated group-specific RBA values.

Figure 1. External variance models.

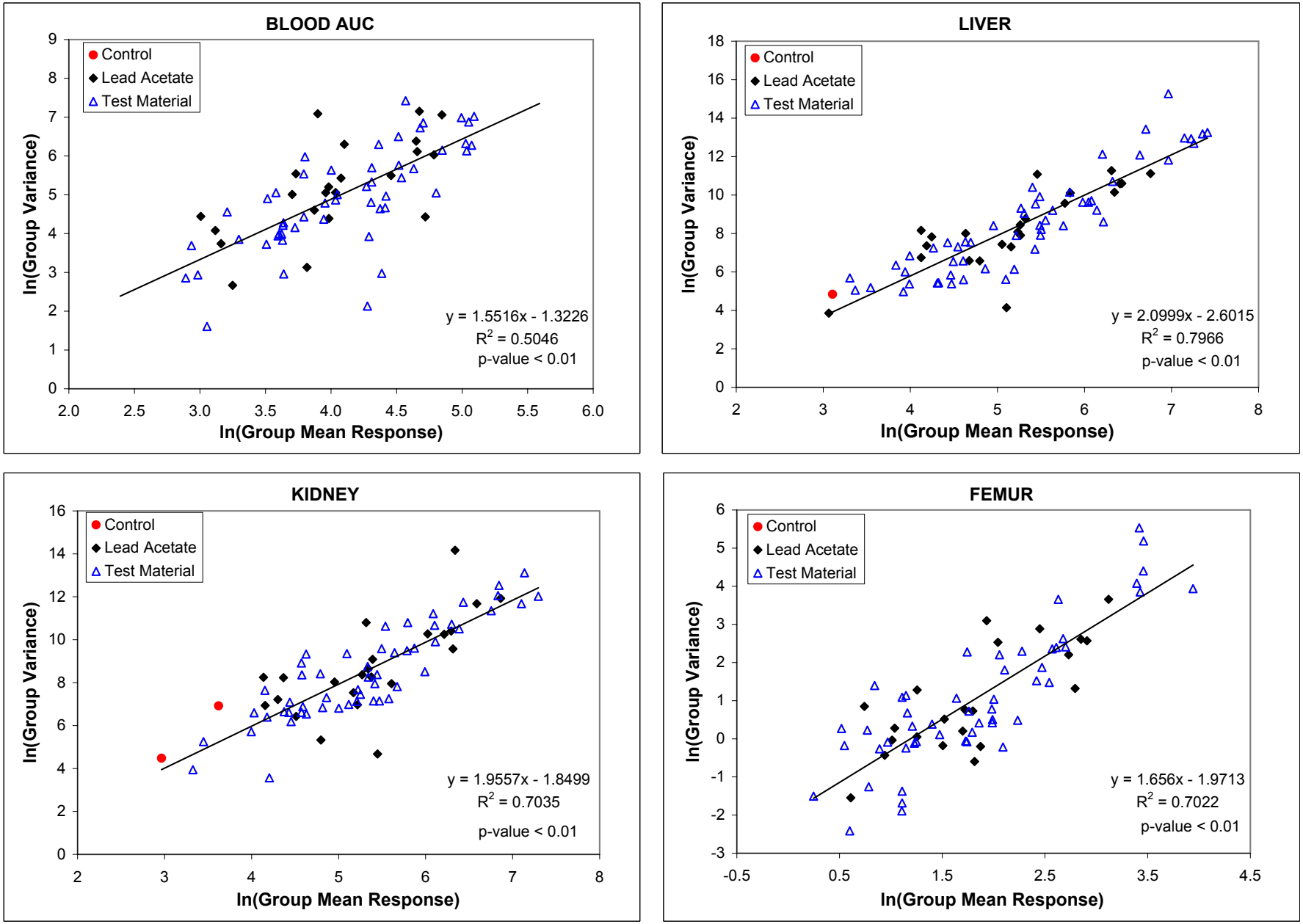
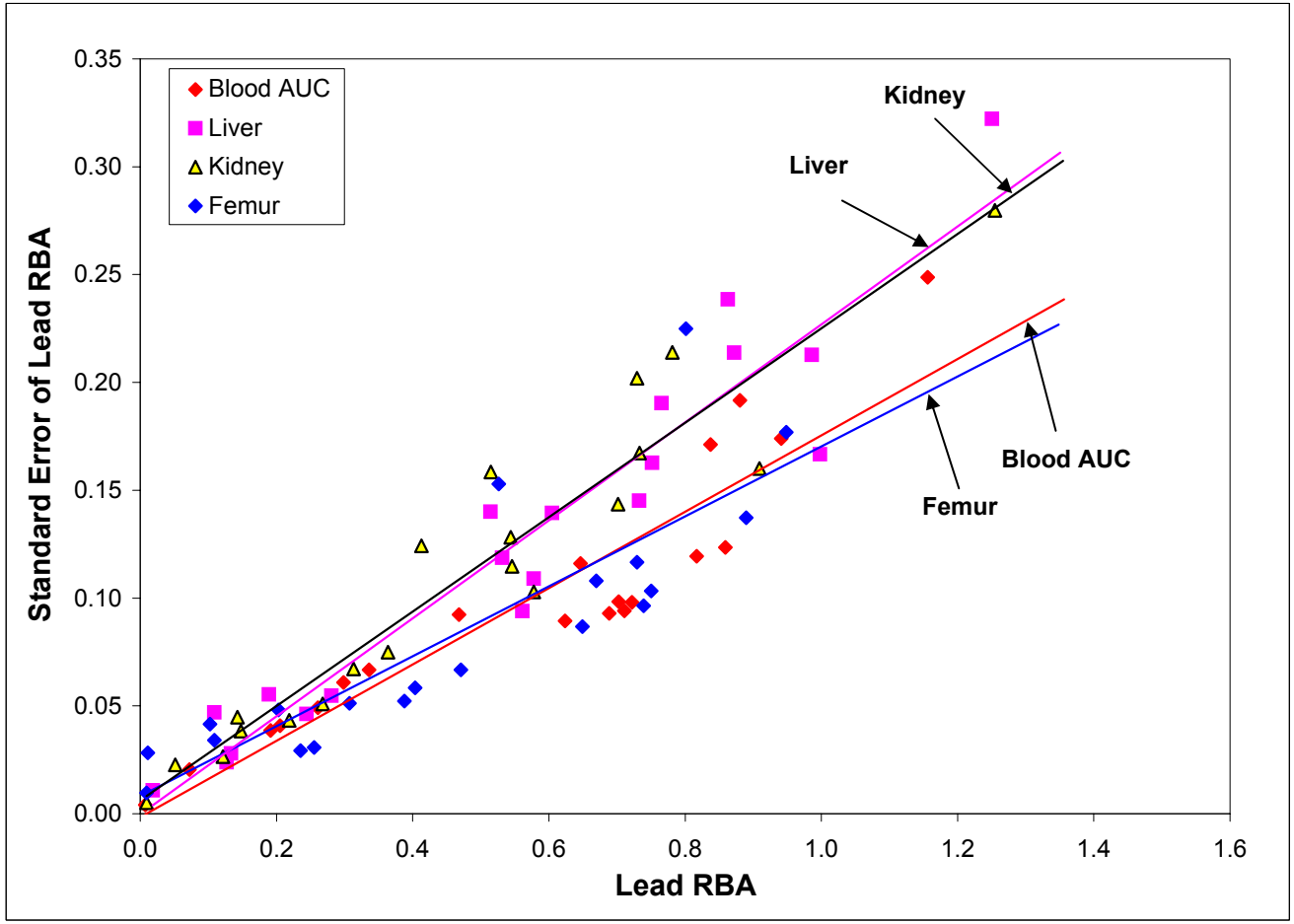


Figure 2. Evaluation of relative precision of measurement endpoints.



Endpoint	Slope	Intercept	R^2
Blood AUC	0.177	-0.002	0.867
Liver	0.227	0.000	0.916
Kidney	0.219	0.006	0.914
Femur	0.162	0.008	0.732

Comparison of Regression Lines	
F	0.638
Fcrit(0.05)	2.227
p	0.699

Figure 3. Example time course of blood lead response.

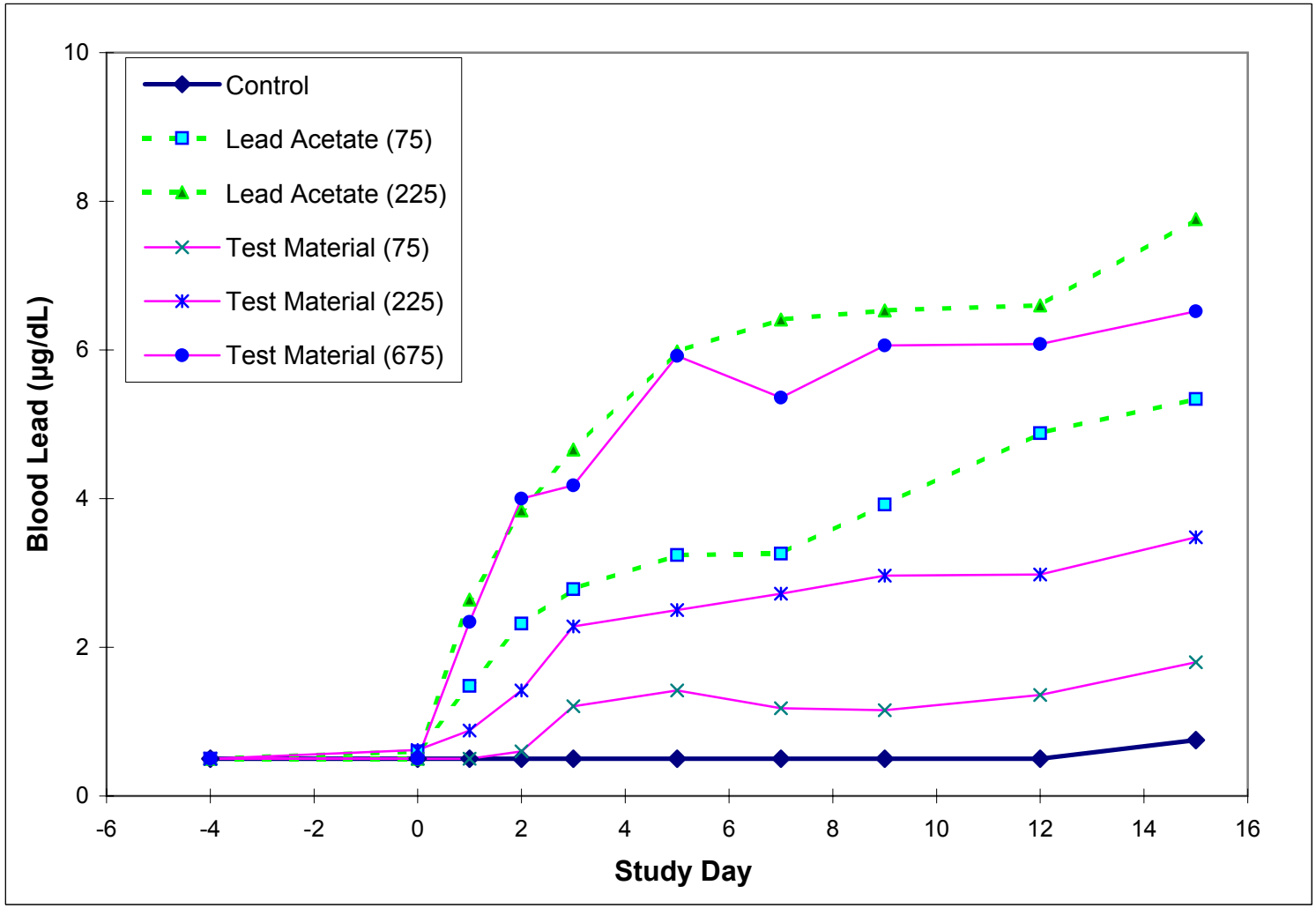


Figure 4. Dose-response curve for blood lead AUC.

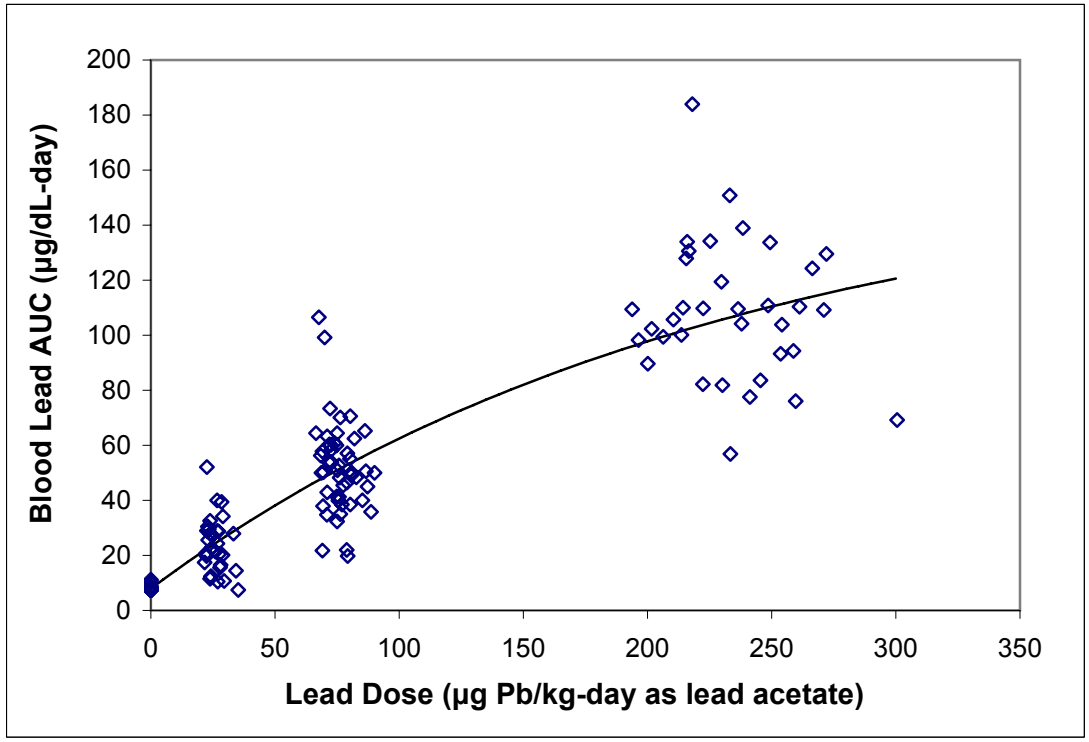


Figure 5. Dose-response curve for liver lead concentration.

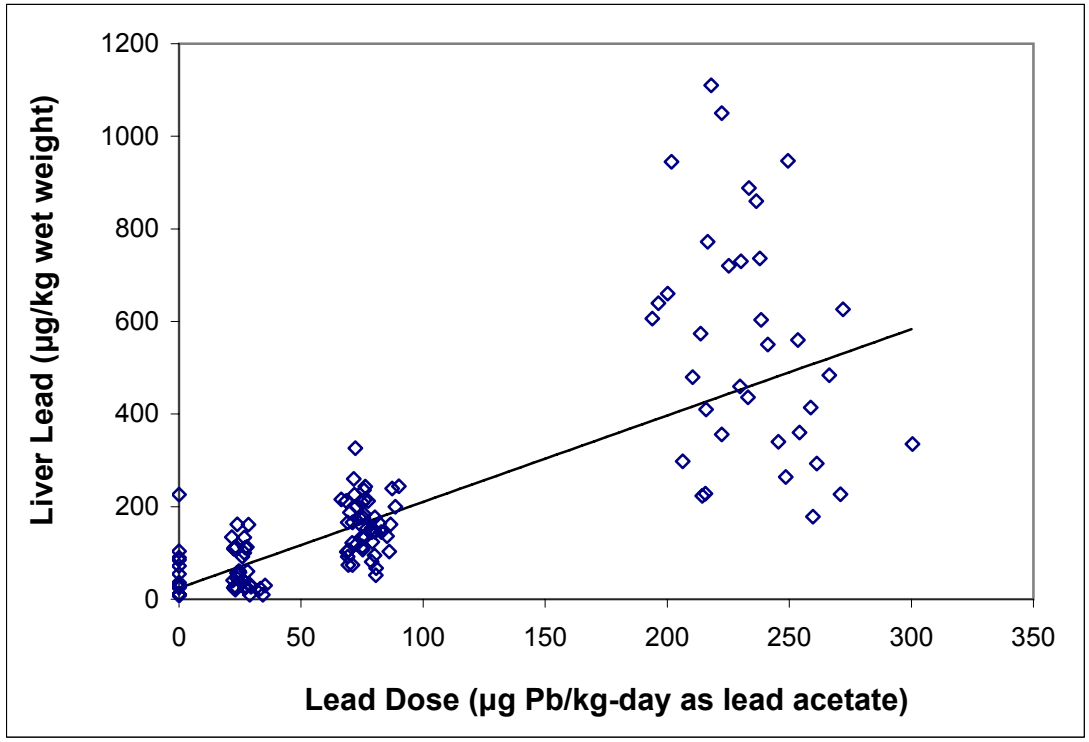


Figure 6. Dose-response curve for kidney lead concentration.

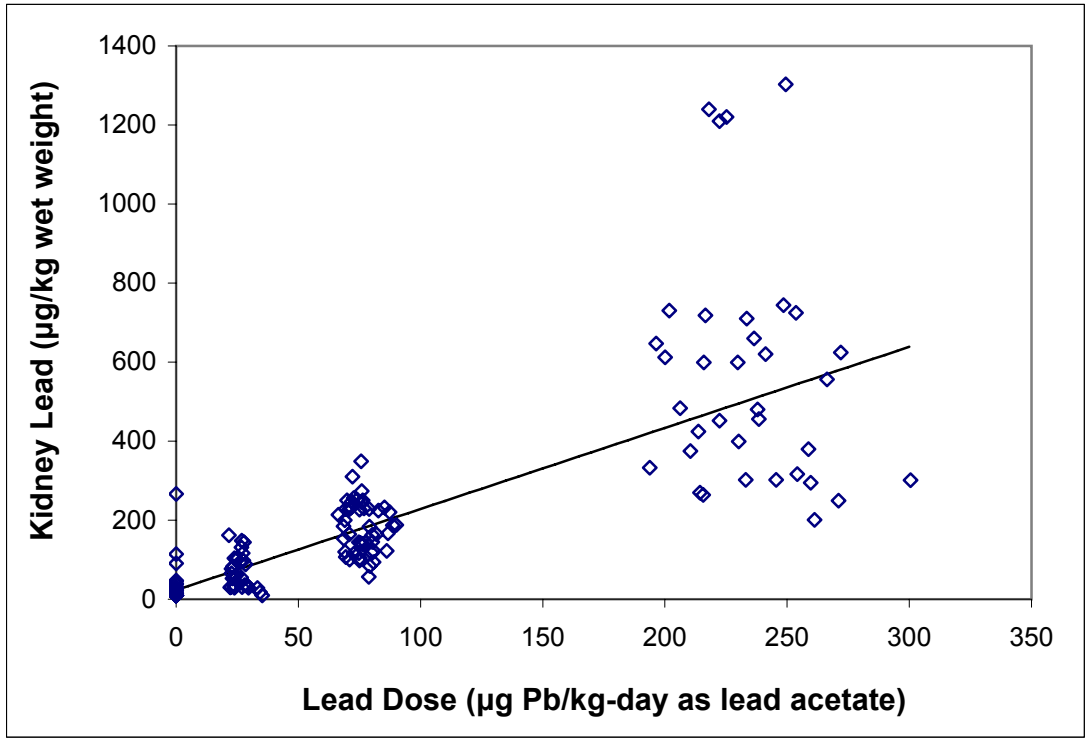


Figure 7. Dose-response curve for femur lead concentration.

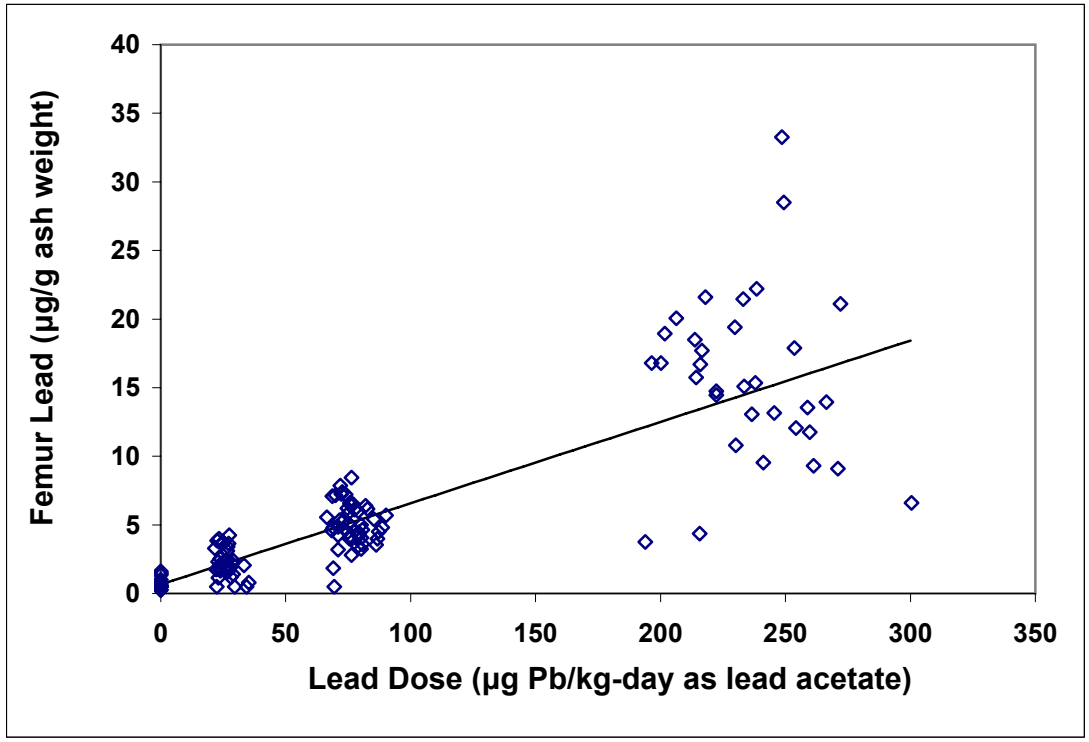


Figure 8. Estimated group-specific lead RBA values.